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PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53 (c).

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TITLE OF THE INVENTION (280 characters)

PROCESS FOR THE QUALITY CONTROL OF MEDICINAL PLANT PRODUCTS

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ENCLOSED APPLICATION PARTS *(check all that apply)*

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Respectfully submitted,
SIGNATURE _____ DATE December 2, 1999

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[ ] Additional inventors are being named on separately numbered sheets attached hereto.

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PROCESS FOR THE QUALITY CONTROL OF MEDICINAL PLANT PRODUCTS

The present invention relates to the quality control of medicinal and nutritional substances derived from plant mixtures. In particular the invention relates to a process for producing a mixture of plant based medicinal or nutritional substances which possesses a pre-defined desired standard, for instance a pharmaceutical grade standard. The invention also allows the origin or quality of a mixture of plant based materials to be determined by comparison with a standard, thereby providing a means for the standardization, quality control, tracking and audit of such mixtures.

Many societies around the world have developed, though the centuries, a system of traditional medicine relying largely on the use of plants and herbs as therapeutic substances. Traditional Chinese medicine and Ayurvedic medicine, practiced for centuries in Asian countries, are examples of well developed systems of plant-based medicine.

In recent years there has been a significant growth of interest amongst the general public in the direct use of plants and plant extracts as heath modifying agents, for instance ginseng, garlic, *Ginkgo biloba, Hypericum* (St John's wort), *Echinacea* and *Aloe vera.* These are currently available on the market as herbal products and dietary supplements and annual sales of these products worldwide are currently in excess of £10 billion. In spite of this marketing potential the mainstream pharmaceutical industry has not so far directed its attention to the development of medicinal products derived from plants. This is due in part to problems associated with the complex nature and inherent non-uniformity of plant materials, including the lack of an established system by which drug regulatory approval for such products can be secured.

The materials used in herbal and plant based medicine are usually whole plants, parts of plants or plant extracts. Since plant materials contain many different chemical components the materials are, by definition, complex mixtures which are difficult to analyse. Many of the remedies employed in traditional Chinese medicine and Ayurvedic medicine mentioned above are mixtures of two or more plant-based components. They are therefore effectively mixtures of mixtures and thus even more difficult to analyse than herbal remedies based on a single plant material. Furthermore, the recipes and methods of manufacture used for such remedies frequently remain undisclosed. These factors make it

very difficult to ensure that two samples of a given remedy, obtained from disparate sources and ostensibly identical, do in fact contain the same mixture of ingredients. This problem, which leads to difficulties in controlling the quality of such materials, has so far limited the acceptability of Eastern herbal remedies to Western herbal practitioners.

The plants used in the practice of herbal medicine are frequently unavailable locally and therefore need to be obtained from sources which are remote from the end user. However, the supply of such plants from remote locations can be erratic and inaccurate, particularly because no detailed monographs including identity and quality standards exist for the plants. The complex mixture of ingredients found in medicinal plants will in any event vary widely in type and concentration depending on many factors including the botanical origin, the location where the plant is grown, the time or year when the plant is harvested and the extraction procedure used. When these plants are in turn mixed with other plants, for instance according to traditional Chinese herbal recipes, there is considerable scope for variability in the resulting product.

As a consequence it is virtually impossible to provide any assurance that a given mixture of plant materials obtained from disparate sources will possess a uniform identity and uniform biological activity. There is no reliable system available at present which both allows the identity and activity of a plant based mixture to be measured against an acceptable standard and is universally applicable to all kinds of plant material.

There is consequently a need for a means of uniquely profiling a given mixture of plant-based materials which will distinguish it from mixtures that are different and yet show it to be consistent with other mixtures that are inherently the same.

The present invention addresses this problem and, in one aspect, provides a process for producing a specified grade therapeutic substance which is derived from, or consists of, a mixture of two or more plant materials, the process comprising:

(i) providing a test sample of the therapeutic substance in the form of a solution or extract;

(ii) generating an NMR fingerprint of the test sample;

(iii) determining whether the NMR fingerprint generated in step (ii) matches that of a pre-determined desired standard sample; and

(iv) selecting the therapeutic substance as being of the specified grade only if

the NMR fingerprint matches that of the said desired standard sample.

The invention resides in the application of high resolution NMR techniques to the characterisation and/or standardisation of mixtures of plant-derived substances. A mixture can be accepted or rejected depending on whether its NMR fingerprint matches that of a pre-determined desired standard sample.

The "desired" standard sample may be, for example, a pharmaceutical grade standard sample or a sample having a defined therapeutic efficacy as established by clinical trials. It may alternatively be a sample of a particular quality, identity or origin. In this context the origin may be, for example, the recipe according to which the mixture was made, the method used for its manufacture, the location(s) where the constituent plant materials were grown and/or the conditions of growing or harvesting of the constituent plant materials.

The invention thus provides a unique descriptor of any given mixture of plant materials which allows that mixture to be objectively determined as being either consistent with, or distinct from, another given mixture without the need to analyse and compare the chemical constituents of the mixtures themselves. In herbal medicine this has the benefit that a given remedy which has been effective in clinical trials can be established as a standard against which other samples of purportedly the same remedy can be compared.

Steps (ii) and (iii) of the process of the invention as defined above are typically carried out by:

(i) submitting the test sample to high field proton NMR and recording one or more NMR spectra;

(ii) evaluating the data obtained from the or each NMR spectrum by one or more computer-based pattern recognition procedures to obtain an NMR fingerprint of the sample; and

(iii) determining the presence or absence of features in the said NMR fingerprint which have been previously identified in the NMR fingerprint of a desired standard sample.

The generation of an NMR fingerprint thus involves a combination of high resolution ^1H NMR at high fields and computer-based pattern recognition procedures (often known as chemometrics). The NMR spectra are typically measured at 400 to 700

MHz and the data derived from them are subjected to statistical analysis by computer programs using techniques such as non-linear mapping and principal component analysis. Examples of high resolution NMR fingerprinting techniques are discussed by M.L. Anthony *et al* in Biomarkers 1996, 1, 35-43 and Molecular Pharmacology 46, 199-211, 1994, and by J.O.T. Gibb *et al* in Comp. Biochem. Physiol. Vol 118B No. 3, pp 587-598, 1997.

In a preferred aspect the process of the present invention comprises:

(i) providing a test sample of a mixture of two or more plant materials in the form of a solution or extract;

(ii) submitting the test sample to high field proton NMR and recording one or more NMR spectra;

(iii) evaluating the data obtained from the or each NMR spectrum by one or more computer-based pattern recognition procedures to obtain a characteristic NMR fingerprint of the sample;

(iv) determining the presence or absence of features in the NMR fingerprint which have been previously identified in the NMR fingerprint of a desired standard sample; and

(v) selecting the said mixture as being of the required grade only if its NMR fingerprint matches that of the said desired standard sample.

An important advantage of this NMR technique is that its not limited by a selective delivery or detection system. Spectra are recorded without prior purification of the test sample, thus allowing all components of the mixture of plant materials to contribute to the overall NMR fingerprint. Analysis by the pattern recognition procedures, or chemometrics, as discussed above reveals potentially valuable features of the spectra which can be used with a high degree of precision in the characterisation of the mixture of plant materials contained in the sample. The analytical techniques used in the pattern recognition procedures take account of the whole NMR spectrum and can incorporate information contained in it which may not be visible to the human eye.

In the process of the invention each plant-derived substance contained in the said mixture typically consists of, or is derived from, a whole plant, a part of a plant, a plant extract or a plant fraction. Preferably each substance consists of, or is derived from, one or

more of the roots, leaves, buds, flowers, fruit, juice and seeds of a plant.

In one aspect of the process of the invention the mixture of two or more plant materials is a Chinese herb product or a mixture of a Western herb product and a traditional Chinese herbal product. Examples of Chinese herbal products include (a) Liu Wei Di Huang Wan, which comprises a mixture of Radix Rehmannia, Fructus Corni, Cortex Moutan, Rhizoma Dioscoreae, Poriae and Rhizoma Alismatis; and (b) Bu Zhong Yi Qi Wan, which comprises a mixture of Radix Astragali, Radix Codonopsis Pilosulae, Radix Glycyrrhizae, Rhizoma Actratylodis Macrocephalae, Radix Angelicae Sinensis, Rhizoma Cimifugae, Radix Bupleuri and Pericarpium Citri Reticulatae. The process of the invention may be used, for instance, to verify that a sample of a traditional Chinese remedy such as Lu Wei Di Huang Wan or Bu Zhong Yi Qi Wan from one source is the same as a sample of the same name obtained from a different source.

The process of the invention as described above relies upon the prior establishment of a specified grade standard for the therapeutic substance in question, which is submitted to high resolution NMR to yield data which are subjected to statistical analysis to provide a characteristic NMR fingerprint. A specified grade standard for a therapeutic substance which is derived from, or consists of, a mixture of two or more plant materials may therefore be provided by a process comprising:

(i) providing a test sample of the therapeutic substance, of the quality desired for the standard, in the form of a solution or extract

(ii) generating a characteristic NMR fingerprint of the test sample; and

(iii) defining the NMR fingerprint obtained in step (ii) as the standard to be met by any sample of the substance which is to be recognised as being of the specified grade.

The desired standard may be as indicated above. Step (ii) typically comprises submitting the test sample to high field proton NMR, recording one or more NMR spectra and evaluating the data obtained from the or each NMR spectrum by one or more computer-based pattern recognition procedures to obtain the NMR fingerprint of the test sample.

In another aspect the present invention provides a process for determining whether a nutritional or therapeutic substance which derives from, or consists of, a mixture

of two or more plant materials has a specified origin or a desired quality, the process comprising:

(i) providing a test sample of the substance in the form of a solution or extract;

(ii) generating an NMR fingerprint of the test sample, for instance as described above;

(iii) determining whether the NMR fingerprint matches that of a previously tested standard sample having the specified origin or desired quality in question; and

(iv) selecting the substance as being of the specified origin or desired quality only if the NMR fingerprint matches that of the said standard sample.

CLAIMS

1. A process for producing a specified grade therapeutic substance which is derived from, or consists of, a mixture of two or more plant materials, the process comprising:

   (i) providing a test sample of the therapeutic substance in the form of a solution or extract;

   (ii) generating an NMR fingerprint of the test sample;

   (iii) determining whether the NMR fingerprint generated in step (ii) matches that of a pre-determined desired standard sample; and

   (iv) selecting the therapeutic substance as being of the specified grade only if the NMR fingerprint matches that of the said desired standard sample.

2. A process according to claim 1 wherein steps (ii) and (iii) are carried out by:

   (i) submitting the test sample to high field proton NMR and recording one or more NMR spectra;

   (ii) evaluating the data obtained from the or each NMR spectrum by one or more computer-based pattern recognition procedures to obtain an NMR fingerprint of the sample; and

   (iii) determining the presence or absence of features in the said NMR fingerprint which have been previously identified in the NMR fingerprint of a desired standard sample.

3. A process according to claim 2 wherein the computer-based pattern recognition procedures include non-linear mapping, principal component analysis and cluster analysis.

4. A process according to any one of the preceding claims wherein each of the plant materials in the mixture consists of, or is derived from, a whole plant, a part of a plant, a plant extract or a plant fraction.

5. A process according to any one of the preceding claims wherein each of the plant materials in the said mixture consists of, or is derived from, one or more of the roots, leaves, buds, flowers, fruit, juice and seeds of a plant.

6. A process according to any one of the preceding claims wherein the said mixture of two or more plant materials is a remedy from traditional Chinese medicine or Ayurvedic medicine.

7. A pharmaceutical grade therapeutic substance or a therapeutic substance having an established clinical efficacy produced by a process as claimed in any one of claims 1 to 6.

8. A process for determining whether a nutritional or therapeutic substance which derives from, or consists of, a mixture of two or more plant materials has a specified origin or a desired quality, the processes comprising:

(i) providing a test sample of the substance in the form of a solution or extract;

(ii) generating an NMR fingerprint of the test sample;

(ii) determining whether the NMR fingerprint matches that of a previously tested standard sample having the specified origin or desired quality in question; and

(iv) selecting the substance as being of the specified origin or desired quality only if the NMR fingerprint matches that of the said standard sample.

9. A process according to claim 8 wherein steps (ii) and (iii) are carried out by:

(i) submitting the test sample to high field proton NMR and recording one or more NMR spectra;

(ii) evaluating the data obtained from the or each NMR spectrum by one or more computer-based pattern recognition procedures to obtain an NMR fingerprint of the sample; and

(iii) determining the presence or absence of features in the NMR fingerprint which have been previously identified in the NMR fingerprint of a desired standard sample.

10. A process according to claim 9 wherein the computer-based pattern recognition procedures include non-linear mapping, principal component analysis and cluster analysis.

11. A process according to any one of claims 8 to 10 wherein each of the plant materials in the said mixture consists of, or is derived from, a whole plant, a part of a plant, a plant extract or a plant fraction.

12. A process according to any one of claims 8 to 11 wherein each of the plant materials in the said mixture consists of, or is derived from, one or more of the roots, leaves, buds, flowers, fruit, juice and seeds of a plant.

13. A process according to any one of claims 8 to 12 wherein the said mixture of two or more plant materials is a remedy from traditional Chinese medicine or Ayurvedic medicine.

14. A process for providing a specified grade standard for a therapeutic substance which is derived from, or consists of, a mixture of two or more plant materials, the process comprising:

(i) providing a test sample of the therapeutic substance, of the quality desired for the standard, in the form of a solution or extract;

(ii) generating a characteristic NMR fingerprint of the test sample; and

(iii) defining the NMR fingerprint obtained in step (ii) as the standard to be met by any sample of the substance which is to be recognised as being of the specified grade

15. A process according to claim 14 wherein step-(ii) comprises submitting the test sample to high field NMR, recording one or more NMR spectra and evaluating the data obtained from the or each NMR spectrum by one or more computer-based pattern recognition procedures to obtain the NMR fingerprint of the test sample.

ABSTRACT

PROCESS FOR QUALITY CONTROL OF MEDICINAL PLANT PRODUCTS

A process for producing a specified grade therapeutic substance which is derived from, or consists of, a mixture of two or more plant materials, the process comprising:

(i) providing a test sample of the therapeutic substance in the form of a solution or extract;

(ii) submitting the test sample to high resolution NMR and generating an NMR fingerprint;

(iii) determining whether the NMR fingerprint generated in step (ii) matches that of a pre-determined desired standard sample; and

(iv) selecting the therapeutic substance as being of the specified grade only if the NMR fingerprint matches that of the said desired standard sample.

The invention thus provides a means for the quality control of medicinal products which consist of a mixture of plants and thereby overcomes problems associated with the inherently complex nature and variable quality of such products.